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Acta Palaeobotanica 56(2): 523–535, 2016
DOI: 10.1515/acpa-2016-0013

Atrotorquata lineata as a proxy for *Juncus roemerianus*, Part I: *Atrotorquata lineata* as a proxy for *Juncus roemerianus* in surface sediments from high-level salt marshes in the southeastern United States

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Received 30 July 2016, accepted for publication 10 November 2016

ABSTRACT. *Juncus roemerianus* is a plant that occurs at the upper reaches of salt water influence in marshes from Delaware to Texas. In 2006 a palynomorphic fingerprint to identify surface sediment from *J. roemerianus* marshes was discovered in a South Carolina study (Marsh 2006, Marsh & Cohen 2008). This fingerprint had four components: (1) high palynomorphic abundance, (2) high palynomorphic diversity, (3) high concentration of Fungal Type A (greater than 10% of the palynomorphs in a given sample) and (4) the presence of the spores of the fungus *Atrotorquata lineata*, which occurred in the sediments of *J. roemerianus* marshes but not in the sediments collected from any other marsh type, even in sediments collected less than a meter away from *J. roemerianus*.

The present study was designed to determine whether (1) *Atrotorquata lineata* occurs in all present-day *Juncus roemerianus* marshes regardless of geographic location within the range of the plant, and whether (2) *A. lineata* is ubiquitous in all surface sediments beneath *J. roemerianus* regardless of the location of the sample in the marsh. As a result of these two findings, *A. lineata* can be considered a proxy for the presence of *J. roemerianus* throughout its range.

To test the first hypothesis, 93 surface samples were obtained from *Juncus roemerianus* marshes throughout the range of the plant (Delaware to Texas). *Atrotorquata lineata* was found in all samples from this range except for those from the northeasternmost end of the range (Virginia and Delaware). A new hypothesis is proposed that temperature may be the factor that explains the loss of the fungal proxy at this northeastern boundary. Furthermore, evidence is presented that geomorphologic and sedimentological factors, such as type of substrate, distance from the ocean, position relative to the shoreline, distance from tidal streams, amount of urbanization, tidal range, or wave fetch, were found to have no impact on the presence or absence of *A. lineata*.

To test the second hypothesis, concerning whether *Atrotorquata lineata* was present in all parts of a *Juncus* stand, surface sediments from a 183 m transect across a monospecific *Juncus roemerianus* marsh were sampled at 15 m intervals. *A. lineata* was found in all samples regardless of position in the stand.

The results of this study show that *Atrotorquata lineata* is omnipresent in sediment from *Juncus roemerianus* marshes throughout all but the most northern edge of the range of *J. roemerianus* and that it was present across the entire extent of a stand of *Juncus roemerianus*. Therefore, *A. lineata* by itself can in fact be considered a proxy for *J. roemerianus*.

KEYWORDS: *Atrotorquata lineata*, *Juncus roemerianus*, Palynology, high level salt marsh, fungal proxy, Southeastern United States

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INTRODUCTION

THE SIGNIFICANCE OF *JUNCUS ROEMERIANUS* STANDS IN COASTAL REGIONS OF THE SOUTHEASTERN UNITED STATES

Juncus roemerianus Scheele is a plant associated with the upper reaches of salt water influence in salt marshes throughout the southeastern United States, as it grows in the transition between fresh and salt water areas, and thus is an indicator of highest tidal inundation. The known geographic range of *J. roemerianus* is from Delaware to Texas, with isolated populations found in Connecticut, New York, New Jersey, Mexico and the Caribbean (Eleuterius 1976) (Fig. 1). *Juncus roemerianus* marshes are generally only intermittently tidally flooded. In the upper reaches especially, they may only be inundated during spring tides and storms.

While it might be assumed that the occurrence of *Juncus roemerianus* marshes could be detected in surface samples by the presence of *J. roemerianus* pollen, *J. roemerianus* pollen is not produced in large numbers because the plant usually spreads vegetatively and not all plants produce flowers. Furthermore, studies by Hodson (1971) and Eleuterius (1975) suggest that only plants near the edge of the colony produce flowers at all. Seedlings are rarely found in nature except at the edges of colonies and in areas that are being newly colonized. The flowers that are produced may be perfect but in many cases the plants are gynodioecious (having female flowers only); no staminate flowers have been found (Seibert 1969, Eleuterius 1978, 1984b). Moreover, while tetrads of *Juncus* pollen can sometimes be found on unprocessed anthers, the small amount of pollen produced by these plants does not seem to be preserved during normal processing to extract pollen (Beecher & Chmura 2004), and no one has ever reported finding *J. roemerianus* pollen in marsh sediments.

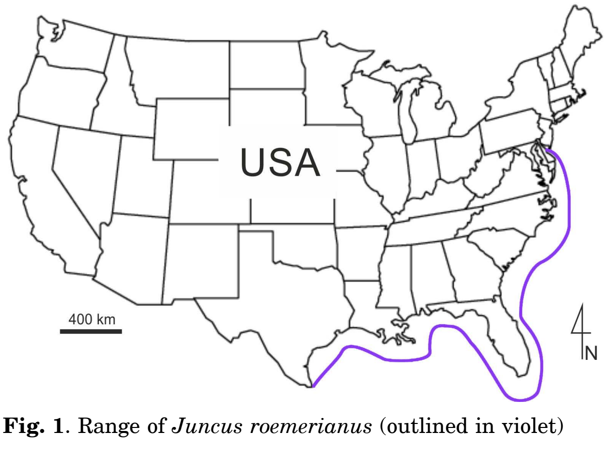

Fig. 1. Range of *Juncus roemerianus* (outlined in violet)

PREVIOUS SALT MARSH STUDIES

There have been many studies of salt marsh environments. Most have focused on identifying physical characteristics such as pH, salinity, inundation time, tidal creek-related topographic and geomorphic changes including rates of accretion and/or erosion of marshes, impacts of rainfall, hydrological modeling, and soil nutrients (e.g. Kurz & Wagner 1957, Hodson 1971, Howard & Frey 1980, Letzsch & Frey 1980, Eleuterius 1984b, Stevenson et al. 1986, Finkelstein & Ferland 1987, Goodbred et al. 1998, Morris et al. 2002, Torres et al. 2003, Pennings et al. 2005, Gardner & Wilson 2006, Torres & Styles 2007, Carter et al. 2008). Other salt marsh studies have focused on the plants, animals, or plankton found in the various salt marsh environments, their impacts on the salt marsh ecosystem and the salt marsh ecosystem's impacts on them (e.g. Seibert 1969, Hodson 1971, Eleuterius 1975, 1978, 1984a, b, 1989, McCraith 1998, Noble et al. 2003, Pennings et al. 2005, Silliman et al. 2005). Additional studies have focused on the microscopic examination of plant fragments found in the surface sediments (e.g. Allen 1977, Cohen & Spackman 1977).

Many other studies have used foraminifera, diatoms, coral, vermetid gastropods, ooids, tree stumps, detrital wood, sediment types, shells, seeds, plant fragments, charcoal and peat found in sediment cores from salt marshes and near-shore environments in an attempt to track changes in accretion, erosion and sea level (including Redfield & Rubin 1962, Scott & Medioli 1978, 1986, Colquhoun et al. 1980, Howard & Frey 1980, DePratter & Howard 1981, Colquhoun & Brooks 1986, Heyworth 1986, Kump & Hire 1986, Laborel 1986, Gayes et al. 1992, Williams et al. 1999, Gardner & Porter 2001, Horton et al. 2006).

Studies of pollen in salt marsh sediments have been undertaken by a number of investigators (Knox 1942, Butler 1959, Sears 1963, Meyerson 1972, Brush & DeFries 1981, Clark 1986, Schneider 1992, Fletcher et al. 1993, Woo et al. 1998, Beecher & Chmura 2004, Ward et al. 2008, Gonzalez & Dupont 2009, Garcia-Moreiras et al. 2015). However, most of these have focused on comparing pollen found in the surface sediment to the vegetation in the surrounding upland areas rather than on the plants in the salt marsh. Upland plants, arboreal species especially, are predominantly wind-pollinated and, as a result, the pollen from these plants can be found in just about any environment within a fairly wide radius of the plants producing them.

Few studies have related the palynomorphs (organic material such as fungal remains, insect parts, algae, and spores in addition to pollen that remains after a sample has been processed to extract pollen; Traverse 1988) found in salt marsh surface sediments with the plants actually occupying the salt marsh. Such studies have been difficult to accomplish, as many salt marsh plants are insect pollinated, so relatively little pollen is produced, and, as previously mentioned, some pollen types are not preserved during processing (Traverse 1988). For example, Beecher and Chmura (2004) found that pollen of *Juncus gerardii* and *J. balticus*, two marine *Juncus* species found in northeastern North America, did not survive the processing procedure, an observation also made by Deng et al. (2006) regarding the pollen of *J. kraussii*, a marine *Juncus* species included in their study of the Whangapoua Estuary in New Zealand. A likely reason for this lack of preservation is that *Juncus* pollen contains a low amount of sporopollenin, the decay-resistant substance that makes up the outer walls of spores and pollen (Traverse 1988). Further reducing the presence of *Juncus* pollen in *Juncus* marsh sediments is the fact that *J. roemerianus* stands, as previously mentioned, tend to reproduce most often vegetatively by rhizomes rather than by pollination and production of seeds (Hodson 1971, Eleuterius 1975). For these reasons, Schneider, who studied salt marshes in Maine, concluded that pollen alone is not sufficient to permit reconstructions of past marsh environments (Schneider 1992). There have been some recent studies that use non-pollen palynomorphs to recreate past environments. While not used in salt marshes, Van Geel & Aptroot (2006) found a relationship between the presence of spores of *Clasterosporium caricinum* and *Carex* and the presence of spores of *Meliola ellisii* as an indicator for local *Calluna vulgaris*. Van der Velden et al. (2015) have related fungal spores and local vegetation assemblage zonation at Lake Challa, Kenya.

A previous study provides the foundation for this current work. This study, described by Marsh (2006) and Mars and Cohen (2006, 2007, 2008), identified the palynomorphic fingerprint of surface sediments taken from *Juncus roemerianus* marshes in South Carolina. Since *J. roemerianus* is a species associated with the upper reaches of salt water influence in salt marshes throughout the southeastern United States, this study provides the background information necessary to investigate the possibility of using the palynomorphic fingerprint to trace sea level changes. The original palynomorphic fingerprint consists of four components: (1) a high number of palynomorphs per sample; (2) high diversity of palynomorph types; (3) a high number of fungal spore Type A, an oval, brown fungal spore frequently appearing to have an indentation in the surface (Fig. 2A); and (4) perhaps most importantly, the presence of the fungal spores, *Atrotorquata lineata*, described below (Fig. 2B, C), which were found in the sediment beneath *Juncus roemerianus*

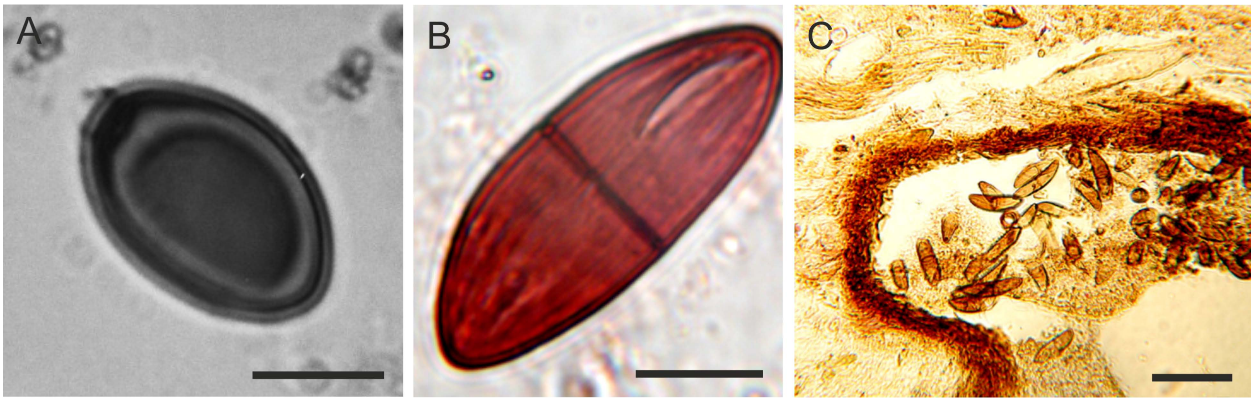

Fig. 2. **A**. Fungal Type A (scale bar: 5 µm); **B**. *Atrotorquata lineata* (scale bar: 10 µm); **C**. Cross section of an ascoma of *A. lineata* with ascospores from a peat core taken in southwest Florida (scale bar: 25 µm); modified from Marsh and Cohen (2008)

plants but not in sediment of other marsh types (low marsh populated primarily by *Spartina alterniflora*, and salt pannes populated primarily by *Salicornia virginica* L.), even when samples were taken just a meter from *Juncus roemerianus* patches.

Atrotorquata lineata was first described by Kohlmeyer and Volkmann-Kohlmeyer (1993). They indicated that *A. lineata* is found in dead standing culms of *Juncus roemerianus*, generally between 11 and 45 cm above the rhizome. The ascospores are 22.8–30.7 µm in length × 7.4–10.5 µm in width, ellipsoidal in shape, and sometimes curved (Fig. 2B and C). They are brown in color and may be constricted at the one septum. They are longitudinally striate and have 5 to 7 germ slits at each apex. Kohlmeyer and Volkmann-Kohlmeyer further suggest that *A. lineata* is probably host-specific to *J. roemerianus*, though there has been one recent report of *A. lineata* being detected in a DNA study of milk from cows grazing in the Italian Alps (Panelli et al. 2013).

PURPOSE AND SCOPE OF THIS STUDY

The purpose of this study was to verify the presence of *Atrotorquata lineata* in sediments from *Juncus roemerianus* marshes throughout the range of the plant and to show that *A. lineata* can be found in all parts of a stand of *J. roemerianus* regardless of the position of the sample within the stand. As previously mentioned, *J. roemerianus* is a plant species associated with the upper reaches of salt water influence in salt marshes throughout the southeastern United States. This study uses the presence of the fungal spores of *A. lineata*, originally identified by Kohlmeyer and Volkmann-Kohlmeyer (1993) and previously described by Marsh (2006) and Marsh and Cohen (2006, 2007, 2008), to identify sediment from high-level salt marshes.

To verify the presence of *Atrotorquata lineata* in surface sediments throughout all parts of a *Juncus roemerianus* stand, surface sediments were collected at 15 m intervals from a 183 m transect across a *J. roemerianus* marsh. Slurry slides were made from these samples and examined microscopically.

To verify the presence of *Atrotorquata lineata* in surface samples throughout the geographic range of *Juncus roemerianus*, a total of 93 surface samples from *J. roemerianus* marshes throughout the range of the plant were obtained. Our method for preparing samples for examination, described below, provides a valid, quick and cheap alternative to expensive and time-consuming palynological processing for determination of the presence or absence of the *J. roemerianus* proxy.

METHODS

SPATIAL DISTRIBUTION AND GEOGRAPHIC EXTENT OF *ATROTORQUATA LINEATA*

In the previously mentioned study (Marsh 2006, Marsh & Cohen 2008) during which the samples were collected that allowed the palynomorphic fingerprint to be established, samples were collected only from the centers of stands of *Juncus roemerianus*. *Atrotorquata lineata* was found in every *J. roemerianus* sample in that study, but, as the samples were taken only from the center of stands, it was not possible to determine from that data whether or not *A. lineata* was indeed present in all parts of a *J. roemerianus* stand from the most inland edge to the most seaward edge.

To address this question, surface samples (top 1 cm of sediment) were collected every 15 m along a 183 m transect through a monospecific stand of *Juncus roemerianus* just off of Crab Haul Creek Road at the Belle W. Baruch Institute for Coastal and Marine Science at Georgetown, South Carolina, from the most seaward edge (Sample CHC 1) to the beginning of upland vegetation (Sample CHC 11). Sample CHC 0 was taken from the *Juncus roemerianus*/*Spartina alterniflora* transition zone at the most seaward end of the transect.

Samples were sent to Global Geolabs in Alberta, Canada, for standard palynological processing and slides made from the residue were examined for the presence of *Atrotorquata lineata* and Fungal Type A.

GEOGRAPHIC RANGE OF *JUNCUS ROEMERIANUS* AND *ATROTORQUATA LINEATA*

The next part of this study was to determine whether *Atrotorquata lineata* is present throughout the entire geographic range of *Juncus roemerianus.*

Samples were obtained throughout the range of *Juncus roemerianus* thanks to researchers from Delaware to Texas. A total of 93 samples from locations in 11 states were examined. Information on sample locations is given below and in Figure 3 and Table 1.

A number of researchers working throughout the range of *Juncus roemerianus* were contacted and asked to provide sediment samples from their local areas (see Table 1 for names of participants, number of samples for each location, and a description of the area sampled when available). Researchers doing the collecting were asked to collect the surface centimeter of sediment from the center of pure stands of *J. roemerianus* and to enclose the samples in either plastic vials or Ziploc bags and send them, along with information

Table 1. Surface samples from throughout the Range of *Juncus roemerianus*

Where collected	Number of samples	Collector	Setting (where known)
Aransas National Wildlife Refuge, Texas	2	Juan Jimenez-Martinez, University of Houston	
Rockefeller Refuge and Lower Breton Sound, Louisiana	2	Sarai Piazza, US Geological Survey	Back-barrier-type environment (RR) and island in the Mississippi Delta (LBS)
Grand Bay National Estuarine Research Reserve, Moss Point, Mississippi	4	Patrick Biber, University of Southern Mississippi	Protected estuarine environment
Grand Bay National Estuarine Research Reserve, Moss Point, Mississippi	6	Mark Woodrey, Grand Bay National Estuarine Research Reserve	
Gulf Coast Research Lab, Ocean Springs, Mississippi	2	Patrick Biber, University of Southern Mississippi	Restored salt marsh in a less protected area near the entrance to a bay
Weeks Bay National Estuarine Research Reserve, Alabama	2	Scott Phipps, Weeks Bay National Estuarine Research Reserve	Estuarine, fetch-limited and relatively protected from storm surges
Appalachee Bay, Wakulla County, Florida	1	Pierre Bourgeois/Thomas Heitmuller, USGS NWRC	
Wacasassa Bay Preserve State Park, Cedar Key, Florida	3	David Hoyt/Rod Hunt, Wacasassa Bay Preserve State Park	
Cape Sable, Florida	1	Arthur Cohen, U. of SC	*Juncus roemerianus* stand surrounded by red mangroves
Fort Clinch State Park, Florida	2	Peter Scalco/Colin Dooley, Fort Clinch State Park	Back barrier/fetch limited environments
Sapelo Island, Georgia	5	Jonathan Garbisch, U GA Mar. Institute	Four samples were located in troughs between Pleistocene shorelines
Cumberland Island, Georgia	3	Frederick Rich, Georgia Southern University	Two samples were on the windward side of the island, relatively unprotected from storms; one was on the leeward side of the island
Georgetown, Charleston, and Beaufort Counties, South Carolina	27	Pamela Marsh/Arthur Cohen, University of South Carolina	Fetch-limited environment on the leeward side of an island (Pleistocene shoreline) (PI) far inland (ca 20 km from the coast) at the upper end of the estuary and in close proximity and surrounded by human development (airport, houses, highway), as such well protected from coastal storms. (IP) inland protected location ca 39 km from the sea (straight line distance, almost 43 km along the estuary), (GC) another protected inland location (9 km straight line and 12.5 km along the estuary to the ocean) near the landward edge of the estuary (JI) landward edge of the salt marsh across from the field laboratory (BI) small patch of marsh surrounded by a housing development (PI) back-barrier site is on the leeward side of an island and is next to housing and a marina and restaurant (MI)
Oregon Inlet, North Carolina	11	Benjamin Horton/Andrew Kemp, U. of Pennsylvania	
Sandy Point, North Carolina	14	Benjamin Horton/Andrew Kemp. U. of Pennsylvania	Landward edge of the island
Assateague National Wildlife Refuge, Maryland/Virginia	4	Jonathan Chase, Assateague National Wildlife Refuge	
Assawoman Wildlife Area, Bethany Beach, Delaware	4	Tracy Elsey, University of Delaware	This location is only ca 1.25 km straight line distance to the ocean behind a barrier beach (and housing development); it is nearly 22 km from the inlet traveling through the estuary

on the location where the samples were collected (latitude/longitude, GPS points, etc.). When samples were received, slurry slides, a new method described below, were made from a portion of each sample, with the rest being held in reserve.

The slurry slides made from these samples were examined microscopically for the presence of *Atrotorquata lineata* using a Leitz Otholux II microscope at 400×.

Fig. 3. Approximate sample locations. Note: Due to scale of map, in some instances more than one sample location is indicated by one star

SLURRY SLIDE TECHNIQUE

For the geographic distribution part of the study, a technique, developed for this study, in which slides are made by mixing a small amount of the sample sediment with glycerin (called the slurry slide technique), was used to produce slides for microscopic examination. While slurry slides are not quite as clear as processed slides, they are quite usable for the purpose of examining samples for the presence of ascospores of *Atrotorquata lineata*, and much faster and cheaper to prepare (Fig. 4). To test the accuracy of slurry slides versus slides prepared by pollen maceration techniques, slurry slides were prepared from material from six representative samples. Duplicate sub-samples of these samples were processed to extract pollen. In all cases, those samples in which *A. lineata* was found in the slurry slides also had *A. lineata* in the pollen-processed slide. Those that did not have *A. lineata* in the slurry slides also did not have it in the processed slides. Slurry slides have several advantages over processed slides. First, slurry slides are much quicker to make than processing a sample to extract pollen. A slurry slide takes only minutes to make, while processing a sample to extract palynomorphs can take up to seven hours, and sending samples to a lab for processing can take two weeks or more. Second, slurry slides are inexpensive to make. The only costs associated with making a slurry slide are the cost of the slide, cover slip, and a drop of glycerin. The costs of processing a slide to extract palynomorphs include the cost of chemicals or the fee charged by a lab to do the work. Third, slurry slides can be made and examined in the field, allowing researchers to know what they have while they are still at the study site. This allows decisions to be made quickly about where and whether to collect more samples.

RESULTS

SPATIAL DISTRIBUTION AND GEOGRAPHIC EXTENT OF *ATROTORQUATA LINEATA*

Ascospores of *Atrotorquata lineata* were present in every sample along the 183 m transect, along with fungal type A. All palynomorphs on each slide were counted to compare with the results of the original study (Marsh 2006, Marsh & Cohen 2008). The number of palynomorphs per slide ranged from 283 to 1271. In general there were fewer palynomorphs in samples farther from the start of upland vegetation, which is to be expected as there are more pollen-producing plants in the upland areas, but there was no true trend of

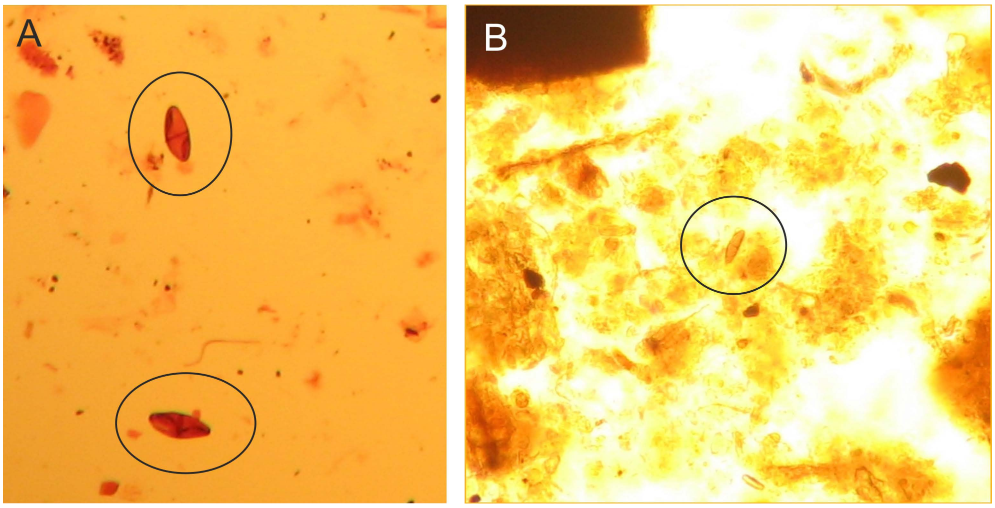

Fig. 4. **A**. A slide in which the sample has been processed to extract palynomorphs. *Atrotorquata lineata* spores are circled; **B**. A slide in which the sample has not been processed to extract palynomorphs (a slurry slide). *Atrotorquata lineata* is circled

decreasing numbers of palynomorphs in more seaward samples. In fact, there was a relative increase in palynomorphs in the sample closest to the ocean.

For better comparison among samples that, as mentioned above, have a wide range of absolute number of palynomorphs per slide, palynomorph abundance was calculated as a percentage of total palynomorphs for each sample. The sample taken from the *Juncus roemerianus*/*Spartina alterniflora* transition zone showed the lowest percentage of both Fungal Type A, at 15.05%, and *Atrotorquata lineata*, at 1.43%. In the samples taken from pure *Juncus roemerianus* stands, fungal type A was present in concentrations of between 19.89% and 48.00% of palynomorphs counted with an average of 29.1%. *A. lineata* was present in samples from pure *J. roemerianus* stands in concentrations ranging from 2.03% to 12.11% (mean 6.72%). This compares quite well to the numbers obtained from the original study that first identified the palynomorphic fingerprint (Marsh 2006), in which the range of fungal type A was from 7.52 to 33.70% (mean 25.20%) and the range for *A. lineata* was 0.64 to 13.71% (mean 4.05%).

SAMPLE LOCATIONS AND DEPOSITIONAL SETTINGS

Sample locations covered the range of *Juncus roemerianus* from Texas to Delaware (Tab. 1). A map of approximate collection locations based on information provided by the researchers and volunteers who provided the samples is found in Figure 3.

The sampling sites represent a wide variety of depositional environments including estuarine bays, near-tidal creek settings, back-barrier marshes, fetch-limited and non-fetch-limited sites, hurricane-impacted sites, human-impacted sites, restored marsh sites, intrashoreline sites and a variety of substrates, tidal ranges, and distances from the ocean.

Texas

Two samples were collected from the Aransas National Wildlife Reserve (ca 24°14′50.21″N, 96°47′25.31″W) on the coast of Texas. These samples were collected at the southern end of the main range of *Juncus roemerianus* in areas that Dr. Juan Jimenez-Martinez described as “patchy” (personal communication). *Atrotorquata lineata* was found in each sample. This is an estuarine, fetch-limited environment.

Louisiana

Two samples were collected at Rockefeller Wildlife Refuge (29°37′9.39″N, 93°24′12.17″W) and Lower Bretton Sound (29°35′25.40″N, 89°36′46.19″W), Louisiana. These samples were obtained from the salt marsh just behind a beach-barrier shoreline. *Atrotorquata lineata* spores were found in each sample.

Mississippi

Samples were collected at the Grand Bay National Estuarine Research Reserve, Mississippi (near 30°22′42.45″N, 88°25′54.73″W) and at the Gulf Coast Research Lab, Mississippi location (near 30°23′34.90″N, 88°47′50.87″W). Dr. Patrick Biber (personal communication) reports that the Gulf Coast Research Lab samples were from restoration sites where plants obtained from nearby areas were planted approximately two years before the samples for this study were collected. The soil there is much sandier than most *Juncus roemerianus* sediments, showing once again that *Juncus roemerianus* can grow in all types of salt marsh soils. Furthermore, although it might be expected that the disturbed sites, later replanted, might not contain *Atrotorquata lineata*, *A. lineata* was found in all samples.

Alabama

Weeks Bay National Estuarine Research Reserve provided two samples from the Weeks Bay NERR location (near 30°23′53.15″N, 87°49′57.82″W). These samples came from highly protected inland fetch-limited sites. *Atrotorquata lineata* was found in both samples.

Florida

Samples from Florida came from Appalachee Bay in the Florida panhandle (30°5′43.76″N, 84°11′5.2″W), Wacasassa Bay Preserve State Park in Cedar Key (29°11′24″N, 82°58′43″W; 29°10′37″N, 82°52′43″W; 29°10′52″N, 82°50′13″W), coastal mangrove swamp in southwestern Florida (near 25°25′57.20″N, 80°08′3.55″W), and Fort Clinch State Park on Florida’s east coast just south of the Florida/Georgia border (30°40′25.2″N, 81°26′8.0″W; 30°41′53.2″N, 81°27′38″W). Unlike all other slides in this part of the study, which were slurry slides, the single southwestern Florida sample was from a slide collection of core samples from a previous pollen study performed by Cohen (1968). *Atrotorquata lineata* was found in this slide and in all other Florida samples.

Georgia

Five samples were obtained from Sapelo Island locations (31°23′37.9″N, 81°16′18.7″W; 31°24′05.7″N, 81°16′57.7″W; 31°24′47.5″N, 81°17′14.6″W; 31°25′11.8″N, 81°17′21.1″W; 31°28′24.4″N, 81°17′6.4″W). Three samples were obtained from St. Catherine's Island (31°35′50″N, 81°9′6.80″W; 31°40′28.27″N, 81°8′ 18.24″W; 31°41′57.86″N, 81°8′47.94″W). *Atrotorquata lineata* was present in each of the samples from both locations.

South Carolina

Surface samples from *Juncus roemerianus* marshes from a wide variety of depositional environments were collected from along the coast of South Carolina as part of the original study to identify the palynomorphic fingerprint (Marsh 2006, Marsh & Cohen 2008). Samples came from Beaufort County (Pinckney Island, (32°16′1.2″N, 80°45′29.88″W; 32°16′1.92″N, 80°45′30.6″W), Inlet Plantation, (32°24′32.4″N, 80°38′28.68″W), and Gardens Corner, (32°36′N, 80°45′38.52″W)), Charleston County (James Island, (32°45′13.68″N, 79°57′45.72″W)), and Georgetown County (The Baruch Institute for Marine and Coastal Science, (33°21′N, 79°12′W), Pawley's Island, (33°24′36″N, 79°7′48″W), and Murrells Inlet, (33°32′60″N, 79°1′12″W)). These sites represented examples of both fetch-limited and non-fetch limited sites as well as greatly urbanized sites and remote, relatively non-impacted sites. Samples from *Salicornia virginica* L. and *Spartina alterniflora* Loisel marshes were also collected from these and other coastal South Carolina sites. Samples from this study were processed to extract pollen and were examined for the presence of the palynomorphic fingerprint. All *Juncus roemerianus* samples contained *Atrotorquata lineata.* Samples taken from *Spartina virginica* and *S. alterniflora* did not. South Carolina samples also included the samples taken along the Crab Haul Creek transect described earlier in this paper. Slides from those samples, as was mentioned earlier, were slurry slides and all of them also contained spores of *Atrotorquata lineata*.

North Carolina

Dr. Benjamin Horton and Andrew Kemp of the University of Pennsylvania provided surface samples from transects across the salt marshes at Oregon Inlet on the Outer Banks of North Carolina (approximately 35°49′10.47″N, 75°33′46.17″W) and Sand Point on Roanoke Island, North Carolina (approximately 35°53′14.89″N, 75°40′29.75″W), as well as a core from Sand Point, which will be discussed in a subsequent section. In each case, all samples identified by Horton and Kemp as "90% *Juncus roemerianus* or 100% *Juncus roemerianus*" contained *Atrotorquata lineata* (personal communication).

Virginia/Maryland

Three samples were provided from Assateague Island National Seashore (exact locations unknown). These samples did not contain *Atrotorquata lineata* spores.

Delaware

Two samples were collected from *Juncus roemerianus* areas in the Assawoman Wildlife Area near Bethany Beach, Delaware (38°30′37.08″N, 75°03′58.32″W). While these samples do appear to be of marine origin, they do not contain *Atrotorquata lineata.*

DISCUSSION

Surface samples were taken at 15 m intervals along a 183 m transect across a *Juncus roemerianus* marsh off of Crab Haul Creek Road at the Belle W. Baruch Institute for Marine and Coastal Science in Georgetown, South Carolina to verify that *Atrotorquata lineata* was present in all parts of a stand of *J. roemerianus*. *A. lineata* was found in every sample from this transect. Additionally, the samples proved to be consistent with those from the original salt marsh study by Marsh and Cohen (2008).

Of the 93 samples examined to determine the geographic range of *Atrotorquata lineata* compared to the geographic range of *Juncus roemerianus*, all but five contained *A. lineata.* Of the five samples that did not contain *A. lineata*, all were collected north of east central North Carolina. Another study is needed to be able to definitively determine why this is so; however, if we disregard the possibility of collection error, there are some hypotheses that might account for the absence of *A. lineata* in *J. roemerianus* marshes north of North Carolina:

1. There are some characteristics of the depositional setting of the Delaware and Assateague Island sample locations (e.g. distance from the ocean, back barrier vs. near shore face, amount of urbanization, tidal range, etc.) that may have caused *Atrotorquata lineata* to be absent.
2. The geographic range of *Juncus roemerianus* plants is greater than the geographic range of the fungus *Atrotorquata lineata.*

Hypothesis 1 does not hold up to close examination. The Delaware samples were taken from a protected area far from the open ocean but so were the Gardens Corner, Inlet Plantation, and James Island, South Carolina samples. The Delaware site was not far from an urban area but the Pawley's Island and Murrells Inlet, South Carolina sites were even closer to development. The Delaware site was in a back barrier location but so were sites at Fort Clinch, Florida and Sand Point, Roanoke Island, North Carolina. Likewise, the Assateague Island samples came from island environments but so did the samples from Sapelo Island and St. Catherine's Island, Georgia, James Island, South Carolina, Lower Bretton Sound, Louisiana, and Sand Point, Roanoke Island, North Carolina. Another possible ecological variable is tidal range. While naturally the tidal range is not available for each sample site, and while frequently the tidal range is less for bays and estuaries than it is for the adjacent ocean, ranges for the reporting stations closest to the sample sites (Tab. 2) show that the Delaware and Assateague sites have tidal ranges in the approximate middle of the overall range of tides for all 93 locations in this study.

In short, there do not appear to be any discrete characteristics of these two sites (distance from the ocean, tidal range, front barrier, back barrier, estuary, island, fetch-limited (as described by Cooper et al. 2007), urbanized or not) that have any impact on the presence of *Atrotorquata lineata.* In samples collected from North Carolina to Texas, if *Juncus roemerianus* is present, so is *A. lineata*. In samples collected north of the North Carolina sites, *A. lineata* does not seem to be present even where *J. roemerianus* is.

Table 2. Mean tidal ranges for stations closest to sample sites. (Data from NOAA Tides and Currents Web Page)

Station name	Tidal ranges [m]
Delaware – Rehoboth Beach	1.19
Maryland/Virginia – Assateague Beach	1.10
North Carolina – Roanoke Sound	0.15
North Carolina – Oregon Inlet	0.61
South Carolina – Murrells Inlet (Marina)	1.35
South Carolina – Pawley's Island (Midway Inlet)	1.34
South Carolina – Baruch Institute (Clambank Creek)	1.44
South Carolina – Folly River	1.66
South Carolina – Pinckney Island	2.20
Georgia – St. Catherine's Island and Sapelo Island	2.10–2.38
Florida – Fernandina Beach	1.83
Florida – Shark River Entrance	0.88
Florida – Cedar Key	0.86
Florida – Apalachee Bay	0.59–0.83
Alabama – Dauphin Island	0.42
Mississippi – Pascagoula	0.42
Louisiana – East Point/Grand Bay	0.32
Texas – Port Aransas	0.40

Hypothesis 2 is much more promising. Any living organism has a range in which it can survive. It is not always the case that the range for a host organism is the same at that of an affiliated species such as a parasite or fungus. While the range of *Juncus roemerianus* is known to be roughly from Delaware to Texas, there is no information available on the range of *Atrotorquata lineata* apart from our own record.

Temperature might be the parameter that controls the boundaries of these two ranges. Comparisons of average low temperature for January from Cape Hatteras, North Carolina (Fig. 5) and the southeastern shore of Maryland, (Fig. 6) the locations closest to the Roanoke Sound/Oregon Inlet and Assateague Beach sampling sites for which these data are available indicate that, while there are year to year variations, the average minimum January (the month with the coldest average minimum) temperature for Cape Hatteras is 6.8°C higher than the average minimum January temperature for the Southeastern Shore of Maryland, with the average from North Carolina being 4°C while the average from southeast Maryland is –2.8°C. This difference may be enough that the more northern sites are out of the temperature range in which *Atrotorquata lineata* can survive. This is a topic for future research.

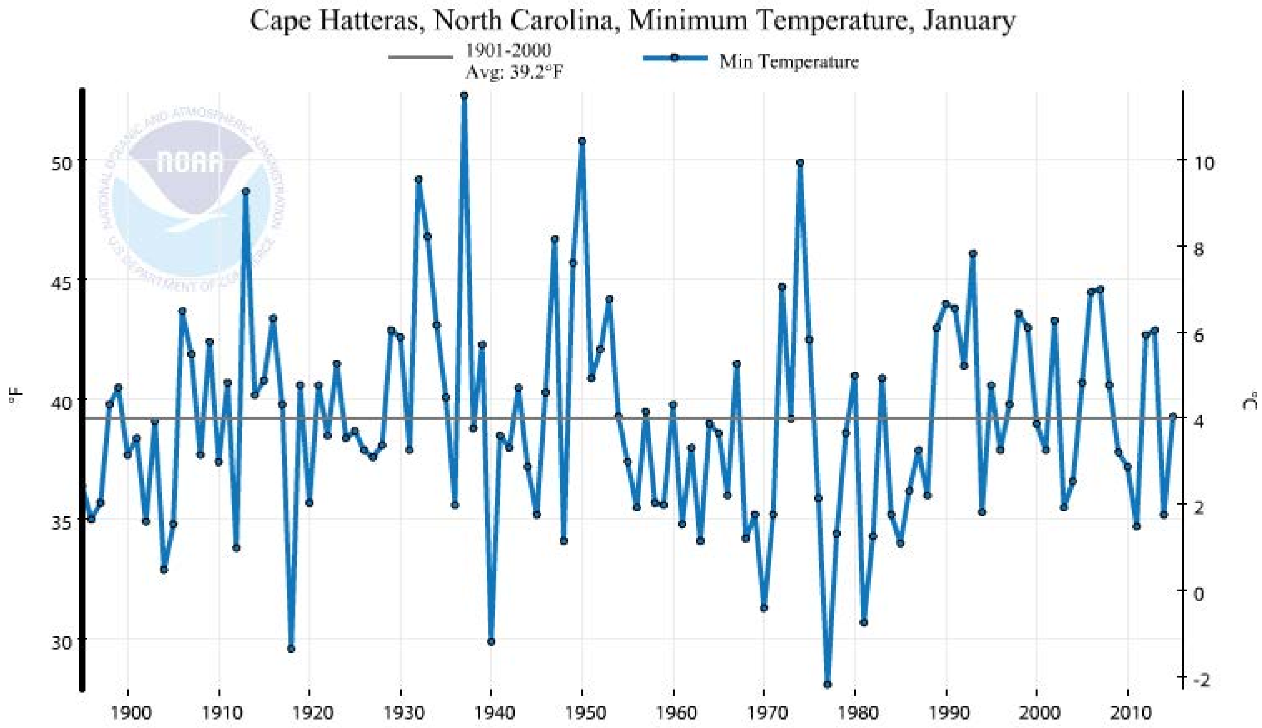

Fig. 5. Minimum average January temperature 1895 to 2015 Cape Hatteras, North Carolina (from http://www.ncdc.noaa.gov/cag/time-series/us)

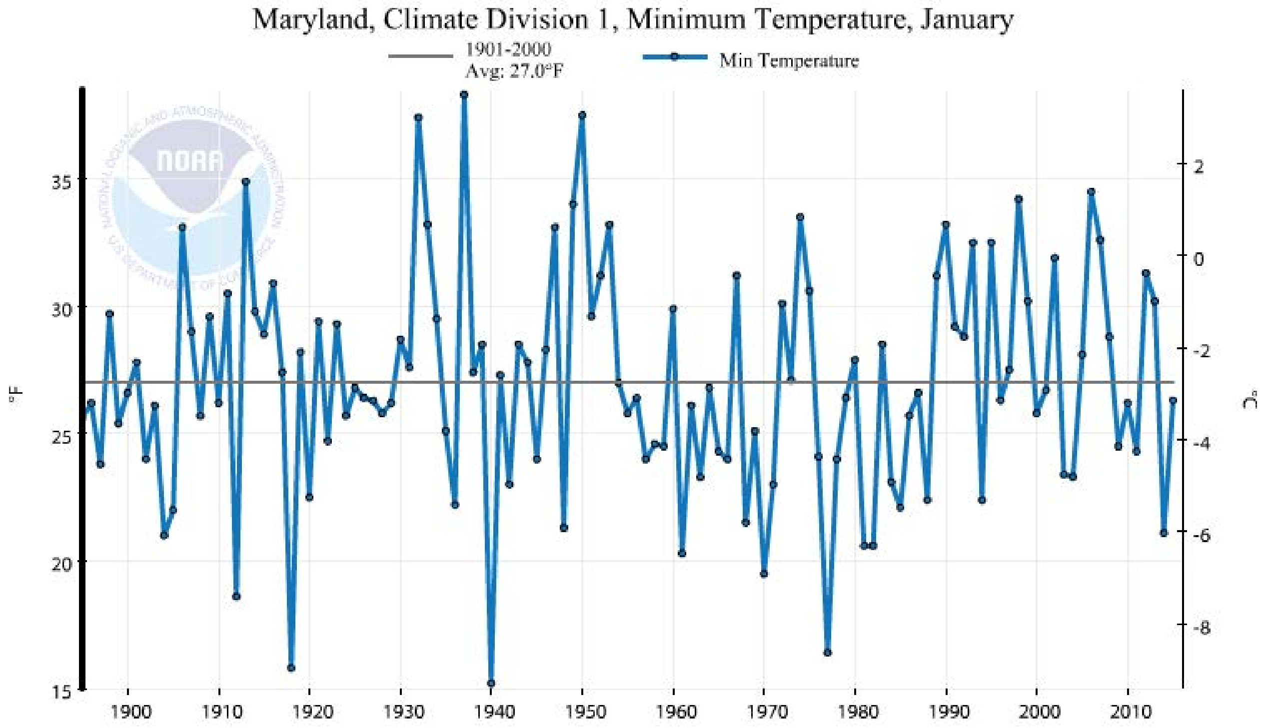

Fig. 6. Minimum average January temperature 1895 to 2015 Southeastern Shore, Maryland (from http://www.ncdc.noaa.gov/cag/time-series/us)

In conducting this research, it was also found that slurry slides made from a small amount of sediment and glycerin provided an inexpensive and quick method of examining sediments, with no loss of accuracy over conventional slides made from sediments that had been processed to extract palynomorphs.

CONCLUSIONS

As a result of this study, the following conclusions can be drawn.

- The palynomorphic fingerprint for *Juncus roemerianus* described by Marsh (2006) and Marsh & Cohen (2008) has been shown to be valid in most of the range of the species. Since the main component of the fingerprint (*Atrotorquata lineata*) occurs only in the culms of *Juncus roemerianus*, *A. lineata* is deposited only in sediment directly beneath the *J. roemerianus* plant. As it is not found in the roots of the plants, and is therefore not found artificially below the surface, it provides a precise record of the position of salt water influence.

- *Atrotorquata lineata* is present in all tested surface sediments from *Juncus roemerianus* marshes from east central North Carolina south. While *J. roemerianus* is found north of this point, temperature may be the limiting factor for *A. lineata*.
- *Atrotorquata lineata* is present in surface sediments across *Juncus roemerianus* stands at any one location.

As a result, we propose that the presence of *Atrotorquata lineata* is indicative of *Juncus roemerianus* and can be used as a proxy for *J. roemerianus* marshes throughout all but the northernmost portion of the range of *J. roemerianus*. As *A. lineata* was also found in a core from Florida radiocarbon-dated at ca 3200 years BP, as well as being found below the surface in an additional study from South Carolina (to be published), we additionally propose that *A. lineata* can be used as a proxy for former positions of *J. roemerianus* marshes and therefore as a marker for sea level change.

Additional work will include testing the temperature limit hypothesis and expanding on using *Atrotorquata lineata* as a marker for sea level variation in areas outside of South Carolina.

ACKNOWLEDGEMENTS

We thank and acknowledge the various researchers around the southeast (listed in Tab. 1) who were kind enough to provide samples for this study and the field assistants who have helped with the South Carolina portion of this study: Rosanna McCroan, Allison Humphries, Melissa Clare Beaty, Carolyn Hudson, Barbara Coleman. We also thank Dr. Bas van Geel of Universiteit van Amsterdam, The Netherlands, and Dr. Mariusz Gałka of Adam Mickiewicz University in Poznań, Poland, for reviewing this manuscript and providing helpful suggestions.

Partial funding for this study came from the South Carolina Sea Grant Consortium, Geological Society of America (Graduate Research Grant), Society of Wetland Scientists South Atlantic Chapter (Student Research Grant), and Baruch Institute (John Hodge Summer Fellowship).

Thanks are due also to the Belle W. Baruch Institute for Coastal and Marine Science and Fort Clinch State Park for sampling permission and to the Department of Geological Sciences at the University of South Carolina for access to lab space and equipment.

REFERENCES

ALLEN E.A. 1977. Petrology and stratigraphy of Holocene coastal-marsh deposits along the western shore of Delaware Bay. The Delaware Sea Grant College Program. University of Delaware. Newark, DE.

BEECHER C.B. & CHMURA G.L. 2004. Pollen-vegetation relationships in Bay of Fundy salt marshes. Can. J. Bot., 82: 663–670.

BRUSH G.S. & DEFRIES R.S. 1981. Spatial distributions of pollen in surface sediments of the Potomac Estuary. Limn. Oceanogr., 26(2): 295–309.

BUTLER P. 1959. Palynological studies of the Barnstable Marsh, Cape Cod, Massachusetts. Ecology, 40(4): 735–737.

CARTER E.S., WHITE S.M. & WILSON A.M. 2008. Variation in groundwater salinity in a tidal salt marsh basin, North Inlet Estuary, South Carolina. Estuar. Coast. Shelf Sci., 76(3): 543–552

CLARK J.S. 1986. Late-Holocene vegetation and coastal processes at a Long Island tidal marsh. J. Ecology. 74(2): 561–578.

COHEN A.D. 1968. The petrology of some peats of southern Florida (with special reference to the origin of coal) Volume 1, Part I: General introduction, petrography, and modern environments. Unpublished PhD Dissertation. The Pennsylvania State University.

COHEN A.D. & SPACKMAN W. 1977. Phytogenic organic sediments and sedimentary environments in the Everglades-mangrove complex: Part II, the origin, description and classification of the peats of southern Florida. Palaeontographica, B, 162: 71–114.

COLQUHOUN D.J. & BROOKS M.J. 1986. New evidence from the southeastern U. S. for eustatic components in the late Holocene sea levels. Geoarchaeology: Intern. J., 1(3): 275–291.

COLQUHOUN D.J., BROOKS M.J., ABBOTT W.H., STAPOR F.W., NEWMAN W.S., & PARDI R.R. 1980. Principles and problems in establishing a Holocene sea-level curve for South Carolina: 143–159. In: Howard J.D., DePratter C.B., & Frey R.W. (eds), Excursions in Southeastern Geology the archaeology-geology of the Georgia coast, Guidebook 20. Geological Society of America. Atlanta, GA.

COOPER J.A.G., LEWIS D.A. & PILKEY O.H. 2007. Fetch-limited barrier islands: overlooked coastal landforms. GSA Today, 17(3): 4–9.

DEPRATTER C.B. & HOWARD J.D. 1981. Evidence for a sea level lowstand between 4500 and 2400 years b. p. on the southeast coast of the United States. J. Sedim. Petrol., 51(4): 1287–1295.

DENG Y., HORROCKS M., OGDEN J. & ANDERSON S. 2006. Modern pollen-vegetation relationships along transects on the Whangapoua Estuary, Great Barrier Island, Northern New Zealand. J. Biogeogr., 33(4): 592–608.

ELEUTERIUS L.N. 1975. The life history of the salt marsh rush, *Juncus roemerianus.* Bull. Torr. Bot. Club, 102(3): 135–140.

ELEUTERIUS L.N. 1976. The distribution of *Juncus roemerianus* in the salt marshes of North America. Chesapeake Science, 17(4): 289–292.

ELEUTERIUS L.N. 1978. A revised description of the salt marsh rush, *Juncus roemerianus.* SIDA, 7(4): 355–360.

ELEUTERIUS L.N. 1984a. Autecology of the black needlerush *Juncus roemerianus.* Gulf Research Reports, 7(4): 339–350.

ELEUTERIUS L.N. 1984b. Sex distribution in the progeny of the salt marsh rush, *Juncus roemerianus*, in Mississippi. Castanea, 49(1): 35–38.

ELEUTERIUS L.N. 1989. Taximetric Analysis of female and hermaphroditic plants among populations of *Juncus roemerianus* under different salinity regimes. J. Coast. Res., 5(1): 29–35.

FINKELSTEIN K. & FERLAND M.A. 1987. Backbarrier response to sea-level rise, Eastern Shore of Virginia, sea-level fluctuation and coastal evolution; Based on a Symposium in Honor of William Armstrong Price. 41: 145–155.

FLETCHER C.H. III, VAN PELT J.E., BRUSH G.S., & SHERMAN J. 1993. Tidal wetland record of Holocene sea-level movements and climate history. Palaeogeogr., Palaeoclim., Palaeoecol., 102: 177–213.

GARCÍA-MOREIRAS I., SÁNCHEZ J.M., & MUÑOZ SOBRINO C. 2015. Modern pollen and non-pollen palynomorph assemblages of salt marsh and subtidal environments from the Ría de Vigo (NW Iberia). Rev. Palaeobot. Palynol., 219: 151–171.

GARDNER L.R. & PORTER D.E. 2001. Stratigraphy and geologic history of a southeastern salt marsh basin, North Inlet, South Carolina, USA. Wetl. Ecol. Manag., 9: 371–385.

GARDNER L.M. & WILSON A.M. 2006. Comparison of four numerical models for simulating seepage from salt marsh sediments. Est. Coast. Shelf Sci., 69(3–4): 427–437.

GAYES P.T., SCOTT D.B., COLLINS E.S., & NELSON D.D. 1992. A late Holocene sea-level fluctuation in South Carolina, Quaternary coasts of the United States: Marine and lacustrine systems. SEPM Spec. Publ., 48: 155–160.

GONZALEZ C. & DUPONT L.M. 2009. Tropical salt marsh succession as sea-level indicator during Heinrich Events. Quatern. Sci. Rev., doi:10.1016/j.quascirev.2008.12.023.

GOODBRED S.L. JR., WRIGHT E.E. & HINE A.C. 1998. Sea-level change and storm-surge deposition in a late Holocene Florida salt marsh. J. Sed. Res., 68(2): 240–252.

HEYWORTH A. 1986. Submerged forests as sea-level indicators: 401–411. In: Van de Plassche O. (ed.), Sea-Level Research: A manual for the collection and evaluation of data. Geo Books. Norwich, UK.

HODSON W.M. 1971. The ecology of *Juncus roemerianus* Scheele in South Carolina. PhD Dissertation. University of South Carolina.

HORTON B.P., CORBETT R., CULVER S.J., EDWARDS R.J. & HILLIER C. 2006. Modern saltmarsh diatom distributions of the Outer Banks, North Carolina, and the development of a transfer function for high resolution reconstructions of sea level. Est. Coast. Shelf Sci., 69(3–4): 381–394.

HOWARD J.D. & FREY R.W. 1980. Holocene depositional environments of the Georgia Coast and continental shelf: 66–134. In: Howard J.D., DePratter C.B., and Frey R.W. (eds.) Excursions in Southeastern Geology, The Archaeology – Geology of the Georgia Coast. Guidebook 20. Geological Society of America. Atlanta, Georgia.

KNOX A.S. 1942. The pollen analysis of the silt and tentative dating of deposits. Robert S. Peabody Foundation for Archaeology, 2: 105–129.

KOHLMEYER J. & VOLKMANN-KOHLMEYER B. 1993. *Atrotorquata* and *Loratospora*: new ascomycete genera on *Juncus roemerianus.* Systema Ascomycetum, 12: 7–22.

KUMP L.R. & HIRE A.C. 1986. Ooids as sea-level indicators: 175–193. In: Van de Plassche O. (ed.), Sea-Level Research: A Manual for the Collection and Evaluation of Data. Geo Books. Norwich, UK.

KURZ H. & WAGNER K. 1957. Tidal marshes of the Gulf and Atlantic coasts of Northern Florida and Charleston, South Carolina. Florida State University Studies, #24. Tallahassee, Florida.

LABOREL J. 1986. Vermetid Gastropods as sea level indicators: 281–310. In: Van de Plassche O. (ed.), Sea-Level Research: A Manual for the Collection and Evaluation of Data. Geo Books. Norwich, UK.

LETZSCH W.S. & FREY R.W. 1980. Deposition and erosion in a Holocene salt marsh, Sapelo Island, Georgia. J. Sed. Petrol., 50(2): 529–542.

MARSH P.E. 2006. Pollen fingerprinting in modern salt marsh environments in South Carolina: the search for analytical standards. Unpublished Master's Thesis. University of South Carolina.

MARSH P.E. & COHEN A.D. 2006. Pollen fingerprinting in modern salt marsh environments in South Carolina: the search for analytical standards. Geol. Soc. Am. Abstracts with Programs, 38(7): 248.

MARSH P.E. & COHEN A.D. 2007. Tracking sea level changes using a newly discovered palynomorphic fingerprint to detect the presence of high-level salt marsh sediments with depth. Geol. Soc. Am. Abstracts with Programs, 39(2): 24.

MARSH P.E. & COHEN A.D. 2008. Identifying high-level salt marshes using a palynomorphic fingerprint with potential implications for tracking sea level change. Rev. Palaeobot. Palynol., 148(1): 60–69.

MCCRAITH B.J. 1998. The distribution and dynamics of fiddler crabs burrowing and its effect on salt marsh sediment composition and chemistry in a southeastern salt marsh. PhD Dissertation. University of South Carolina.

MEYERSON A.L. 1972. Pollen and paleosalinity analyses from a Holocene tidal marsh sequence, Cape May County, New Jersey. Mar. Geol., 12: 335–357.

MORRIS J.T., SUNDARESHWAR P.V., NIETCH C.T., KJERFVE B. & CAHOON D.R. 2002. Responses of coastal wetlands to rising sea level. Ecology, 83(10): 2869–2877.

NOAA National Centers for Environmental Information Webpage. URL: http://www.ncdc.noaa.gov/cag/time-series/us. Accessed May 22, 2015.

NOAA Tides and Currents Webpage. URL: http://tidesandcurrents.noaa.gov/index.shtml. Accessed May 8, 2007.

NOBLE P.A., TYMOWSKI R.G., FLETCHER M., MORRIS J.T. & LEWITUS A.J. 2003. Contrasting patterns of phytoplankton community pigment composition in two salt marsh estuaries in southeastern United States. App. Envir. Microbiol., 69(7): 4129–4143.

PANELLI S., BRAMBATI E., BONACINA C. & FELIGINI M. 2013. Diversity of fungal flora in raw milk from the Italian Alps in relation to pasture altitude. SpringerPlus, 2: 405.

PENNINGS S.C., GRANT M.B. & BERTNESS M.D. 2005. Plant zonation in low-latitude salt marshes: disentangling the roles of flooding, salinity and competition. J. Ecol., 93(1): 159–167.

REDFIELD A.C. & RUBIN M. 1962. The age of salt marsh peat and its relation to recent changes in sea level at Barnstable, Massachusetts. Proceedings of the National Academy of Sciences of the United States of America., 48(10): 1728–1735.

SCHNEIDER Z.I. 1992. Relationships between modern vegetation and pollen deposition of tidal marshes at Wells, Maine, U.S.A. Master's Thesis. University of Maine, Orono.

SCOTT D.S. & MEDIOLI F.S. 1978. Vertical zonations of marsh foraminifera as accurate indicators of former sea levels. Nature, 272: 528–531.

SCOTT D.S. & MEDIOLI F.S. 1986. Foraminifera as sea level indicators: 435–456. In: Van de Plassche, O. (ed.), Sea-Level Research: A Manual for the Collection and Evaluation of Data. Geo Books. Norwich, UK.

SEARS P.B. 1963. Vegetation, climate and coastal submergence in Connecticut. Science, New Series, 140(3562): 59–60.

SEIBERT R.W. 1969. Flowering patterns, germination, and seed and seedling development of *Juncus roemerianus*. PhD Dissertation. North Carolina State University at Raleigh.

SILLIMAN B.R., VAN DE KOPPEL J., BERTNESS M.D., STANTON L.E, & MENDELSSOHN I.A. 2005. Drought, snails, and large-scale die-off of southern US salt marshes. Science, 310: 1803–1806.

STEVENSON J.C., WARD L.G. & KEARNY M.S. 1986. Vertical accretion in marshes with varying rates of sea level rise: 241–259. In: Wolfe D.A. (ed.) Estuarine Variability. Acad. Press. New York,.

TORRES R. & STYLES R. 2007. Effects of topographic structure on salt marsh currents. J. Geophys. Res., 112. F02023, doi:10.1029/2006JF000508.

TORRES R., MWAMBA M.J. & GONI M.A. 2003. Properties of intertidal marsh sediment mobilized by rainfall. Limnol. Oceanogr., 48(3): 1245–1253.

TRAVERSE A. 1988. Paleopalynology. Unwin Hyman. Boston.

VAN DER VELDEN D., VERSCHUREN D. & LOUWYE S. 2015. Calibration of fossil non pollen palynomorphs as a palaeovegetation proxy. Geol. Soc. Am. Abstracts with Programs, 47(7): 61.

VAN GEEL B. & APTROOT A. 2006. Fossil ascomycetes in Quaternary deposits. Nova Hedwigia, 82: 313–329.

WARD L.G., ZAPROWSKI B.J., TRAINER K.D. & DAVIES P.T. 2008. Stratigraphy, pollen history and geochronology of tidal marshes in a Gulf of Maine estuarine system: climatic and relative sea level impacts. Mar. Geol., 256(1–4): 1–17.

WILLIAMS K., EWEL K.C., STUMPF R.P., PUTZ F.E. & WORKMAN T.W. 1999. Sea-level rise and coastal forest retreat on the west coast of Florida, USA. Ecology, 80(6): 2045–2063.

WOO H.J., OERTEL G.F. & KEARNEY M.S. 1998. Distribution of pollen in surface sediments of a barrier-lagoon system, Virginia, USA. Rev. Palaeobot. Palynol., 102: 289–303.